

What is claimed is:

*all of nature's
pairs identified!*
1. A method of amplifying an amplification sequence of a target nucleic acid sequence comprising:

*are pairs? if so,
hybridized? if so,
may not hybridize
complementary
complementary
if some sequence
of target sequence
is strong & plan
self - complementary
must complementary
can be
incompletely
hybridized*
(a) contacting said amplification sequence with a plurality of pairs of amplification probes, wherein the member probes of each of said pairs of amplification probes are complementary to each other and at least one same hybridizing member of each pair of probes is also complementary to a portion of said amplification sequence. *→*)

15 all other pairs
(b) allowing said hybridizing members of said amplification probes to hybridize to a different portion of said amplification sequence, with said amplification probes binding to said amplification sequence in a contiguous manner;

20 steps →
(c) causing said hybridized amplification probes to join together to form an amplification product;

*25 the gene only contains
many complementary
copies of part of*
(d) effecting separation of said amplification product from said target sequence; and,

30
(e) repeating steps (a) through (d).

2. The method of claim 1 wherein said

*hybridized amplification probes are joined together by
the action of an enzyme.*

*3. The method of claim 2 wherein said enzyme
is a ligase.*

4. The method of claim 1 wherein said hybridized amplification probes are joined together through a chemical reaction.

*Value 5
independent*
5. The method of claim 1 wherein at least three ^{complement} ~~segments~~ pairs of amplification probes are used.

✓ 6. A method for detecting a nucleic acid sequence having three or more ligated nucleic acid 10 segments comprising:

✓ 15
(a) contacting said nucleic acid sequence with two detection probes, wherein each of said detection probes is complementary to a portion of each of two of said ligated nucleic acid segments which are adjacently situated in said nucleic acid sequence;

✓ 20
(b) allowing each of said detection probes to hybridize to two adjacently situated segments of said nucleic acid sequence, with said detection probes binding to said nucleic acid sequence sufficiently adjacent to each other to enable an interaction to occur between said hybridized detection probes;

✓ 25
(c) detecting the presence of said hybridized detection probes.

✓ 30
7. The method of claim 6 wherein at least one of said detection probes is labeled.

✓ 35
8. The method of claim 7 wherein one of said detection probes is labeled with a first proximity label and the other of said detection probes is labeled with a second proximity label.

9. The method of claim 6 further comprising:

see 13
5 and,
see cl 6
how detect?
not enzyme
enzymatic
reaction
ligase

(a) causing said hybridized detection probes to join together to form a ligated detection product;

(b) detecting the presence of said ligated detection product.

10. The method of claim 9 wherein said hybridized detection probes are joined together by the action of a enzyme. *ligase*

15 11. The method of claim 10 wherein said enzyme is a ligase.

20 12. The method of claim 9 wherein said hybridized detection probes are joined together through a chemical reaction.

25 *7.33*
in absence
and not
enzymatic
(labeled)
and antibody)

13. The method of claim 9 wherein one of said detection probes is labeled with a detectable label and the other of said detection probes is labeled with a means for removing said ligated detection product from solution.

30 *see 13*
detect (e)
detect

14. A method for detecting a target nucleic acid sequence which may be present in a test sample comprising:

35 (a) contacting said test sample with a plurality of pairs of nucleic acid amplification probes, wherein the member probes of each of said pairs of amplification probes are complementary to each other and at least one *same* hybridizing member of each pair of probes is also complementary to an amplification sequence of said target nucleic acid sequence;

*how can more
than one member
hybridize to the
amp. seq. f(6)*

(b) allowing said hybridizing members of said amplification probes to hybridize to a different portion of said amplification sequence, with said amplification probes binding to said amplification sequence in a contiguous manner;

5 (c) causing said hybridized amplification probes to join together to form an amplification product;

10 *for
say* (d) effecting separation of said amplification product from said amplification sequence;

15 *(see cl 6
(except 3...)
no anticipated loss
for amp. prod. segments)* (e) contacting said amplification product with two detection probes, wherein each of said detection probes is complementary to a portion of each of two of said amplification probe segments which are adjacently situated in said amplification product;

20 (f) allowing each of said detection probes to hybridize to two adjacently situated segments of said amplification product, with said detection probes binding to said amplification product sufficiently adjacent to each other to enable an interaction to occur 25 between said hybridized detection probes;

30 (g) detecting the presence of said hybridized detection probes.

*only have 1
hybridized probe* 35. The method of claim 14 further comprising:

(a) causing said hybridized detection probes to join together to form a ligated detection product; and,

(b) detecting the presence of said ligated detection product.

16. The method of claim 14 wherein said hybridized amplification probes are joined together by the action of an enzyme.

17. The method of claim 15 wherein said enzyme is a ligase.

10

18. The method of claim 14 wherein said hybridized amplification probes are joined together through a chemical reaction.

15 19. A reagent for use in the amplification of an amplification sequence comprising a plurality of pairs of nucleic acid amplification probes, wherein the member probes of each pair of amplification probes are complementary to each other and at least one same hybridizing member of each pair of amplification probes is also complementary to a given portion of said amplification sequence, with the nucleic acid sequences of each pair of amplification probes selected to be complementary to a different portion of said amplification sequence, the amplification probes being capable of hybridizing to the amplification sequence in a contiguous manner sufficiently adjacent to each other to enable the probes to be joined together.

20. A reagent for use in the detection of a nucleic acid sequence having three or more ligated nucleic acid segments comprising two nucleic acid detection probes, wherein each of said detection probes is complementary to a portion of each of two of said ligated nucleic acid segments which are adjacently situated in said nucleic acid sequence, with at least

25

30 35

ref 80, 3
or more, or N/A?
see cl 6
(S) 30

see cl 6
(S) 30

one of said detection probes being provided with a label, the detection probes being capable of hybridizing to said nucleic acid sequence sufficiently adjacent to each other to enable an interaction to occur between 5 said detection probes.

✓ 21. A kit for use in the detection of a target nucleic acid sequence which may be present in a test sample comprising:

10

(a) a plurality of pairs of amplification probes, wherein the member probes of each pair of amplification probes are complementary to each other and at least one same hybridizing member of each pair of amplification probes is also complementary to an amplification sequence of said target nucleic acid sequence, with the nucleic acid sequences of each pair of amplification probes selected to be complementary to a different portion of said amplification sequence, said 20 amplification probes being capable of hybridizing to said amplification sequence in a contiguous manner sufficiently adjacent to each other to enable the probes to be joined together to form an amplification product; and,

25

(b) two detection probes, wherein each of said detection probes is complementary to a portion of each of two amplification probe segments of said amplification product which are adjacently situated in said amplification product, with at least one of said detection probes 30 being provided with a label, said detection probes being capable of hybridizing to said amplification product sufficiently adjacent to each other to enable an interaction to occur between said hybridized detection probes; <

35

add
B7